

Development and standardization of an improved type 1 diabetes genetic risk score for use in newborn screening and incident diagnosis

Seth A. Sharp, Stephen S. Rich, Andrew R. Wood, Samuel E. Jones, Robin N. Beaumont, James W. Harrison, Darius A. Schneider, Jonathan M. Locke, Jess Tyrrell, Michael N. Weedon, William A. Hagopian^{##}, Richard A. Oram^{##}

Corresponding Authors

Richard A. Oram
RILD Level 3
University of Exeter Medical School
Royal Devon and Exeter NHS Foundation Trust
Barrack Road
Exeter EX2 5DW UK
r.oram@exeter.ac.uk
+44 (0) 1392 406819

William A. Hagopian
Pacific Northwest Research Institute
720 Broadway
Seattle WA 98122 USA
wah@uw.edu
+1 (206) 726 1200

Abstract

Objective

Previously generated genetic risk scores (GRSs) for type 1 diabetes (T1D) have not captured all known information at non-HLA loci and particularly at HLA risk loci. We aimed to more completely incorporate HLA alleles, their interactions and recently discovered non-HLA loci into an improved T1D GRS2 to better discriminate diabetes subtypes and to predict T1D in newborn screening studies.

Research Design and Methods

We used 6481 cases and 9247 controls from the Type 1 Diabetes Genetics Consortium (T1DGC) to analyse variants associated with T1D in both the HLA region and across the genome. We modelled interactions between variants marking strongly associated HLA haplotypes and generated odds ratios to create an improved T1D GRS2. We validated our findings in UK Biobank. We assessed the impact of the T1D GRS2 in newborn screening, diabetes classification, and to provide a framework for comparison to previous scores.

Results

The T1D GRS2 used 67 single nucleotide polymorphisms (SNPs) and accounted for interactions between 18 HLA DR-DQ haplotype combinations. The T1D GRS2 was highly discriminative for all T1D (AUC=0.92; $p<0.0001$ vs older scores) and even more discriminative of early onset T1D (AUC=0.96). In simulated newborn screening, The T1D GRS2 was nearly twice as efficient as HLA genotyping alone, and 50% better than current genetic scores in general population T1D prediction.

Conclusion

An improved T1D GRS2 is highly useful to classify adult incident diabetes type, and to improve newborn screening. Given the cost-effectiveness of SNP genotyping, this approach has great clinical and research potential in T1D.

Introduction

Type 1 diabetes (T1D) involves autoimmune destruction of insulin producing pancreatic beta cells. While prominent in childhood, it may present at any age (1). Measurement of islet autoantibodies (IA) in venous blood can reveal active disease years before the clinical diagnosis (2). Early, pre-clinical identification of T1D can minimize morbidity, medical costs at clinical onset, and might facilitate prevention therapy (3). However, IA surveillance is expensive and difficult in young children. Better selection of high risk infants will improve the cost-effectiveness of any prediction program (3-5). Further, IA measurement in incident cases of adult diabetes is not sufficiently sensitive (6) nor specific (7) for accurate classification of diabetes subtype, the latter important for appropriate treatment (8-10).

T1D has a large heritable component, evidenced by a twin concordance rate of up to 70% (11) and a sibling risk of ~8% (12). The majority of risk is explained by variation at a few very strongly associated loci including the HLA class 2 DR-DQ loci, the HLA class 1 region, and >50 associated non-HLA single nucleotide polymorphisms (SNPs) including INS and PTNP22 (12). This high genetic heritability creates the potential for powerful diagnostic discrimination if the majority of genetic risk for T1D can be captured (13; 14). We and others have shown incorporation of multiple T1D risk loci into an integrated genetic risk score (GRS) is more powerful than HLA DR-DQ genotyping alone in the discrimination of T1D with scores incorporating 10-40 SNPs demonstrating a discriminative ROC AUC of 0.84-0.87(4; 15-17). Additionally, the simplification of T1D genetic risk as a continuous variable allows non-HLA scientists and clinicians much easier access to T1D genetic information for a variety of clinical and research uses.

To date, published genetic risk scores have not fully captured all information on risk of T1D. HLA class 2 DR-DQ haplotypes (at the IDDM1 locus) are by far the most important factor in heritable T1D risk (18; 19). Full assessment of HLA DR-DQ risk is complex due to the association of many common haplotypes with T1D, and the non-additive interactions between them (19-21). Our original T1D GRS (16), herein referred to as T1D GRS1, included the susceptible HLA haplotypes DR3-DQ2 and

DR4-DQ8 and the resistant haplotype DR15-DQ6, but missed many other DR-DQ haplotypes important in T1D genetic risk or protection. Advances in the density of SNP arrays combined with ever larger sets of reference data (such as 1000 Genomes) have led to improvements in the accuracy of both genome-wide and HLA imputation, thereby allowing for more precise measurement of HLA-associated T1D risk. Better tagging of HLA risk using SNPs that can be cheaply and rapidly genotyped offers the possibility to capture T1D genetic risk associated with HLA DR-DQ more comprehensively into a T1D GRS2.

In this study we aimed to improve SNP-capture of HLA DR-DQ risk, including haplotype interactions. We also aimed to improve the GRS by capture of additional non-DR-DQ loci via additional SNPs found to be associated with T1D risk. We generate a new T1D GRS2 with this information. We assess the additional discriminative power from this approach, demonstrate how the improvement might enhance clinical applicability, and create a framework within which different GRSs may be compared.

Methods

We used HLA and genome-wide imputation of a large T1D case-control dataset (22) to assess and improve capture of HLA and non-HLA T1D-associated loci into an improved GRS2. We first focused on IDDM1, the HLA DR-DQ locus which is characterized by strong linkage disequilibrium and unusually frequent polymorphisms. The extensive HLA-T1D literature with published odds ratios were used to select, among common DR-DQ haplotypes, those that conferred significant risk or protection for T1D. This list comprised many more haplotypes than those included in previously published T1D GRS. To call haplotypes in our samples we imputed HLA alleles and used correlation statistics to identify tagging SNP variants. We further analyzed the relationship of specific combinations of two DR-DQ haplotypes (haplogenotypes). We next analyzed the impact of HLA alleles outside of DR-DQ, and of additional published T1D risk loci across the rest of the genome. Finally, we combined variants into a genetic risk score and validated our results using the approach shown in **Supplementary Figure S1**. To differentiate from our original T1D GRS (16), we refer to the score based on the new combination of loci and haplotypes as “T1D GRS2”.

Research Subjects: Type 1 Diabetes Genetic Consortium To investigate genetic associations, we used the T1DGC ImmunoChip case-control genetic data with ~164,000 genotyped variants (22). The classification of T1D was made based on clinical features previously described. The study consisted of a collection of white Caucasian cases and controls (6670 T1D cases, 9416 controls) combined from previous studies such as the UK WTCCC. Cases ranged from those with a clinical diagnosis before age 1 to a maximum age of 16 years. The mean age of diagnosis was 7.75 years.

Research Subjects: UK Biobank To validate our results, we used the UK Biobank Affymetrix Axiom Array data with ~821,000 variants genotyped in a subset of ~374,000 individuals identified as European Caucasian by genetic clustering

methods (23). Cases were defined by a strict set of criteria favoring specificity among selected individuals for T1D rather than sensitivity to include all T1D cases:

- Clinical diagnosis of diabetes at or below age 20
- On insulin within 1 year from the time of diagnosis
- Still on insulin at the time of recruitment
- Not using oral-antihyperglycemic agents
- Did not ever self-report as having type 2 diabetes

The total number of T1D cases fitting these criteria was 387. We also identified 11,885 cases of type 2 diabetes (T2D) by a similarly strict definition:

- Clinical diagnosis of diabetes at age 35 or older
- Not on insulin within 1 year from the point of diagnosis
- Did not ever self-report as type 1 diabetic
- Excluded if diagnosed within last 12 months as not able to check insulin usage.

Imputation We used the 1000 Genomes reference panel to impute a total of 80.6 million variants in the T1DGC discovery data. We used data which was centrally imputed by UK BioBank to a combination of the Haplotype Reference Consortium (HRC) panel and the UK10K + 1000 Genomes panels (23). To impute HLA alleles in the T1DGC dataset, we used SNP2HLA (24) with the T1DGC reference panel consisting of 5225 SNP-genotyped and HLA-genotyped North Americans. We imputed a total of 424 alleles at the following loci: HLA-A/B/C/DQA1/DRB1/DQB1/DPB1. Alleles were imputed to 4-digit resolution and average imputation accuracy was high (mean info $r^2=0.977$).

HLA DRB1-DQA1-DQB1 haplotypes Using imputed T1DGC data we identified every possible combination of DRB1, DQA1 and DQB1 alleles occurring on the same haplotype. Each haplotype present in $\geq 0.5\%$ of cases and controls was evaluated as a predictor of T1D in a logistic regression model adjusted for every possible haplotype. Common haplotypes not observed to be independently associated ($P>0.05$) but with suspected interaction effects were also included in further analysis. We used HLA imputation data from a subset of ~128,000 UK Biobank samples to

identify variants that would best mark these haplotypes from their correlation r^2 and d' statistics.

Interaction modeling To assess whether interactions existed between 136 possible DR/DQ haplotype pairings (haplogenotypes) we generated multiplicative interaction terms from genotype dosage values. Logistic regression, with the interaction term as the independent variable and each haplotype of the pairing included as a covariate, was used to test the strength of interaction. A Bonferroni-corrected cutoff ($P < 3.7 \times 10^{-4}$) was applied. Interaction terms below the P value threshold identified haplotype combinations with evidence of interaction.

Additional loci A total of 323 imputed alleles from HLA loci outside DR/DQ were tested in a logistic regression adjusting for the DR/DQ haplotypes previously identified – this was necessary as long-range linkage is well known to occur in the HLA region between class I, class II and class III loci. A Bonferroni-corrected threshold was applied ($P < 1.6 \times 10^{-4}$). HLA alleles that remained significant after adjustments for both DR/DQ and for multiple testing were added to the model. Outside of the HLA region, we included variants from our GWAS of the T1DGC data in addition to variants identified in previously published GWAS.

Generating the GRS We took the natural log of each odds ratio identified in the discovery data to generate a beta statistic for each pair of haplotype combinations and each allele. For haplogenotypes with an identified interaction each person was assigned a single score at the DR/DQ locus. For haplogenotypes with no interaction identified the score was generated from the sum of each allele weighted by its beta. The remaining loci were then added to the total score by multiplying the number of risk alleles by the beta for each variant. Odds ratio and betas were coded to correspond to the minor allele for HLA loci as this tagged the presence of an allele subtype. Positive beta values therefore imply a risk increasing effect from an allele and negative a risk decreasing effect. An equation summarizing generation of the score is presented in [Supplementary Figure S2](#).

Statistical methods Logistic regression, with T1D as the outcome and variants of interest as independent variables, was used in discovery data to identify and validate associations and generate odds ratios. Covariates were included as described above.

A linear mixed model, as implemented in BOLT-LMM (25), was used to perform GWAS of all imputed variants in the discovery data with type 1 diabetes status as the outcome. Covariates included were principal components and sex. Variants from T1D GRS1 were included in further conditional analysis. Simulations of population screening performance in both GRS and HLA typing were based on an assumed T1D prevalence of 0.3% and sensitivity and specificity derived from the UK Biobank dataset.

We tested the ability of the GRS to discriminate cases from controls in T1DGC and replicated our findings in UK Biobank by using the area under the curve (AUC) of the receiver operator characteristic (ROC). ROC statistics were generated with DeLong's algorithm. The Youden Index was calculated as $j = \text{sensitivity} + \text{specificity} - 1$. To compare the discriminative power of different GRS we used a χ^2 test on a subset containing all T1D cases and 24,000 randomly selected controls. Bonferroni correction was used where appropriate to account for multiple testing.

Results

Genome-wide association analysis of T1DGC cases and controls identified additional significant T1D genetic associations not captured by T1D GRS1 both within and outside of HLA (Figures 1B, 1E). We assembled genetic components into a new risk score, T1D GRS2, grouping them as either a) HLA-DRB1-DQA1-DQB1 alleles, b) genes in the HLA region but distinct from DR-DQ, and c) genes outside the HLA region. Our approach to T1D GRS2 generation is summarized in Supplementary Figures S1 and S2.

Improved capture of HLA risk We identified tag SNPs for 14 HLA DQA1-DQB1 haplotypes (11 more than for T1D GRS1) associated with T1D in the T1DGC case-control cohort ([Supplementary Table S1](#)). We chose to study SNP variants linked to DQ haplotypes rather than DRB1 for three reasons. First, DQ has a higher impact on T1D risk than DR. Second, most DQ haplotypes were each linked to only a single DRB1 allele in T1DGC anyway. Third, due to low frequency in the imputation panel, we were unable to discriminate all DRB1 subtypes known to impact T1D risk, such as DRB1*04:0X. Once DQ haplotypes were identified, we sought to detect and use their interactions to refine our estimates of T1D risk. [Supplementary Table S2](#) illustrates a heat map of DQ haplotype combinations (haplogenotypes) odds ratios based on imputed DQ haplotypes in T1DGC. From DQ tag SNPs, we identified 18 DQ haplotypes with significant interaction terms and generated interaction-specific odds ratios ([Supplementary Table S3](#)). For less common haplogenotypes with insufficient data to estimate interaction odds ratios ($n < 50$ cases in T1DGC) or where no significant interaction was identified, we generated ORs for the individual haplotypes in remaining case-control data ([Supplementary Table S1](#)). This approach resulted in an HLA Class II (IDDM1) component of the genetic risk score derived from 14 HLA SNPs covering most major T1D associated Class II haplotypes and specific odds ratio terms for 18 haplogenotype combinations.

We identified 21 further HLA region SNPs not representing DR-DQ haplotypes that each associated independently with T1D ([Supplementary Table S4](#)). Some mark known T1D associated alleles at Class I A, B or C, or at Class II DP, while others were not correlated to any specific HLA gene but rather located near the XL9 regulatory region between DRB1 and DQA1 or in presumed regulatory regions near DRA1 or BTNL2 (26; 27).

The combined model of 35 total HLA-region SNPs was much more discriminative of T1D than the 5 SNP model in T1D GRS1, in both the T1DGC discovery dataset (ROC-AUC 0.907 vs 0.856; $p < 0.0001$) and UKB validation set (ROC-AUC 0.897 vs 0.865 ($p < 0.0001$)) ([Figures 2A and 2D](#)).

Improved capture of non-HLA genetic risk We then identified 32 non-HLA loci (summarized in (28)) which again each associated independently with T1D

(Supplementary Table S5). Taken together, these non-HLA SNPs improved discrimination of T1D compared to GRS1 in the T1DGC Discovery set ROC AUC 0.715 vs 0.699, $p < 0.001$) and in the validation dataset (ROC AUC 0.75 vs 0.707, $p < 0.001$) (Figure 2E). The improved discrimination was most clearly observed in the validation data due to the denser SNP array and better imputation of non-HLA variants.

A T1D GRS2 using 67 SNPs offers significantly improved prediction of T1D

The final combined T1D GRS2 utilized a total of 67 SNPs (14 DR-DQ, 21 other HLA and 32 non-HLA SNPs) and markedly improved discrimination of T1D vs. T1D GRS1. The ROC curve AUC increased from 0.886 to 0.927, $p < 0.0001$ in the T1DGC Discovery dataset and from 0.893 to 0.921 in the UK BioBank validation $p < 0.0001$ (Figures 2C and 2F). Genome-wide association analysis of the T1DGC cases and controls using T1D GRS2 as a conditional variable demonstrated little remaining genome-wide significant SNP associations, indicating vastly improved capture of T1D-associated information (Figure 1).

Assessment of T1D GRS against diabetes type We then compared the ability of GRS1 and the new T1D GRS2 to separate known T1D from the background population in UK Biobank. For example, using the optimal T1D GRS2 cutoff (at maximum Youden Index) allowed inclusion of 82.7% of T1D patients while 88.5% of the background population was properly excluded (Figure 3B). In a similar exercise comparing T1D vs T2D patients, T1D GRS2 alone excluded 87.8% of T2D patients but included 82.7% of T1D patients (Figure 3D). Both represent substantially increased specificity at similar sensitivity vs T1D GRS1 (Figures 3A and 3C).

Using the T1D GRS2 to predict future T1D among infants

Simulations show the T1D GRS2 could be used for newborn screening to select babies requiring follow-up for islet autoantibody (AAB) surveillance (Table 1, top). Individuals with a T1D GRS2 $> 90^{\text{th}}$ centile in a general population contain $> 77\%$ of cases of future T1D with a T1D risk of 2.4%, those with a score of $> 99.9^{\text{th}}$ centile have a T1D risk over $> 20\%$, but this cut off would only identify 7% of future T1D cases. Comparative simulations of general population screening demonstrated T1D

GRS2 predictive performance much better than T1D GRS1 (Table 1, middle), but most importantly more than twice as good (less than half as many children requiring AAB surveillance) compared to prior methods using only HLA DR-DQ (Table 1, bottom).

Assessment of T1D GRS2 against age of diagnosis Stronger genetic risk of T1D is known to associate with younger age of diagnosis (17; 29). We tested the correlation of GRS2 against onset age within T1DGC (Supplementary Figure S3), and demonstrated especially improved performance at very young onset ages ($p=7 \times 10^{-44}$ Pearson's correlation). The effect appeared to be explained by greater HLA risk in those children (HLA-only $p=6 \times 10^{-39}$, non-HLA $p=1 \times 10^{-4}$).

Comparative performance with other genetic scores: To date, a variety of genetic scores have been described and used in T1D. Genetic scores vary according to SNPs available from array data, cost and sample size limitations within a study, and the methods of score calculation. By analyzing different scores against a reference population (either a background population or a cohort with T1D) it is possible to compare different scores based on centiles in the reference set to compare the sensitivity and specificity of each score. In Table S6 the Youden's Index is used to compare performance of several recently published GRS used in T1D. A Youden Index of 1 describes perfect discrimination at a particular threshold. The T1D GRS2 had the highest Youden index (0.698) across a range of centiles and the highest ROC-AUC ($P < 0.0001$ vs all scores). The Youden's index value as UKB centile varies is plotted for multiple GRS scores in Supplementary Figure S2.

Discussion

We combined 67 SNPs into an improved T1D genetic risk score termed T1D GRS2. With improved capture of HLA DR-DQ risk, HLA interactions and additional non-HLA SNPs, the T1D GRS2 included all independent genome-wide significant T1D risk from T1DGC ImmunoChip data. When directly compared with other genetic scores used to date, T1D GRS2 significantly improved discrimination of T1D from T2D and

controls. Indeed, whilst all published T1D GRS, derived by a variety of methods, have had ROC AUC's of 0.86 or greater (4; 15-17), GRS2 yielded the greatest T1D discrimination with an AUC ROC of 0.93 in the T1DGC dataset. This in large part resulted from including many more HLA features in GRS2 compared to prior scores (4; 15; 16).

We used T1D GRS2 to compare the proportion of screened newborn infants in a population who must be followed after screening to capture differing proportions of future T1D cases. As an example, the detection of 77% of future cases would require following 20.9% of screened infants using only HLA DR-DQ selection criteria, 14.3% of infants using our T1D GRS1, but only 9.5% of infants using T1D GRS2 (Table 1 and Supplementary Table S6) representing a major cost savings. Each iteration represents a critical improvement in cost-effectiveness for general population pediatric T1D prediction strategies. More stringent cut-off points can identify very high risk pediatric populations suitable for trials of primary prevention therapies, such as the 0.1% of a population with >10% absolute disease risk. This approach is already being tested (4) and improved selection of high risk individuals using scores like T1D GRS2 will only enhance these efforts.

Discrimination of T1D from T2D cases can help classify incident diabetes cases in adulthood, where incorrect classification is common and can lead to incorrect treatment, greater medical costs and greater morbidity (8; 10). T1D GRS2 improved discrimination between incident T1D and T2D patients in a similar manner to that between T1D and healthy controls (Figure 3). This is expected due to the very minimal genetic overlap between T1D and T2D. Our previous findings (16) suggest that a combined model using onset age, AAB status and BMI will improve discrimination of incident cases much further. A similar approach of combining genetic and non-genetic risk successfully predicted progression rate to T1D in at-risk relatives in the TrialNet Pathway to Prevention study (combining AAB data, the T1D GRS1, age and a metabolic score) (30). A logical next step is to use the T1D GRS2 in similar settings to assess discriminative power when combined with these additional predictors of T1D.

The T1D GRS2 was most predictive of T1D in very early life, consistent with existing genetic data (17; 29). At very young ages there is less additional risk data available (such as AAB and BMI) to combine with genetic risk, yet unexpected T1D onset can carry particularly high morbidity and even mortality (31). A powerful GRS may be clinically useful in this setting to identify people at risk of mortality and morbidity from T1D in early childhood. Our estimates of cases captured assumes equivalent genetic risk across all childhood, and T1D GRS2 may be even more sensitive and specific to identify very young onset T1D patients.

Our T1D GRS2 development approach captured multiple HLA effects. Previous T1D genetic scores captured the commonest and best described HLA class 2 DR3-DQ2 and DR4-DQ8 risk alleles (4; 15; 16), a single protective allele (DR15-DQ6) (16) and two class 1 alleles (HLA A*24, HLA B*57) (4; 16). We now capture many more of the DR-DQ haplotypes, nearby regulatory regions, more Class I alleles, and HLA DP alleles, that confer susceptibility or resistance to T1D (20; 32). We worked to ensure that GRS2 captured interaction effects between these haplotypes. Complex interactions in the Class 2 HLA region have previously been described (21) but have not been integrated into methods for T1D prediction. We also discovered T1D-associated HLA region SNPs not tracking classic HLA alleles but in intergenic regulatory regions between DRB1 and DQA1 which regulate Class II gene expression, e.g. near XL9 and BTNL2. These have been described in the context of SLE (26) and Sarcoidosis (27) but are not well characterized in T1D. Similar conditional approaches to ours may yield further mechanistic insights, however investigating these further is outside the scope of this work. Future more dense arrays, sequencing, and analyses of gene expression are likely to better characterise these regions, their interactions and potential mechanisms. Finally, T1D GRS2 covered many more HLA-Class I and HLA-DP alleles known to affect T1D risk (32). One limitation was that we struggled to capture DRB1*04 subtypes. These are currently not well imputed, and we were unable to find SNP tags that distinguished the high risk DR4 alleles from protective DRB1*04:03 and DRB1*04:07 alleles on the DQA1*03:01-DQB1*03:02 background. Use of more dense SNP arrays and better imputation reference sets to achieve this goal may improve future GRS performance.

The amount of genetic information incorporated into a T1D genetic risk score that can be easily used in the clinic and for research must necessarily represent a balance between effective cost of implementation and maximisation of genetic information. The most genetic information could theoretically be obtained from using whole genome sequencing (WGS) which is currently not feasible, or from genomic risk scores that use all genetic association information, unfiltered for genome wide association significance. Genome wide risk scores have recently been very effective at improving prediction of non-HLA linked disease (33), but the extremely strong HLA bias of T1D heritability, and the inability of these models to currently analyze the HLA region, means that these methods are less suitable for T1D prediction. At the other end of the spectrum, SNP-based GRS can vary from containing all associated T1D SNPs to smaller numbers of SNPs such as the previously described 10-SNP version of GRS1 (16). We believe that whilst it is difficult to envisage a WGS approach to population-wide screening, the falling cost of SNP typing, and the very strong association of T1D with a few SNPs in the HLA region makes a more comprehensive GRS a practical choice for use in such population-based research and public health settings.

A limitation of T1D GRS2 is its use of genetic information discovered and validated in European Caucasian cohorts. We currently lack large well described case-control cohorts of other ethnicities. Initial data from Perry et al (17) and others suggest that the T1D GRS2 will be discriminative in Hispanics, but possibly less so in Africans, although our own unpublished work (Oram, Weedon and Yajnik personal communication) suggests that the score works well in South Asian populations. Larger datasets examining genetic associations in these populations are required to fully define the utility of genetic risk scores in prediction and classification of T1D. Additionally, it is possible that even in populations of similar ethnic background, different environments might mediate different genetic associations requiring score adjustment. However, there is currently little evidence for strong gene-environment effects in T1D. These limitations of stratification, and the assumptions made when

summing genetic risk into a score, must be weighed against ease of use and translatability. A single T1D GRS2, not fully adjusting for ethnicity or environmental interactions may capture most genetic risk while avoiding the increased complexity necessary in more customized genetic risk scores. We and others are working to develop and improve GRS for use in diverse populations customized for ethnicity and race (17; 34).

A further limitation of our T1D GRS2 is its origin in T1DGC ImmunoChip data that is itself limited in SNP coverage and density. As increasingly powerful and up-to-date SNP arrays become more widely used, and broader reference datasets such as TOPMED (35) allow higher quality imputation, the sensitivity to more directly identify causal variants, rare T1D risk alleles, and complex interactions will likely improve GRS even further. However the step-change in improved prediction in our study is utilizing new information from the most important region in T1D genetics and the current predictive power of the T1D GRS2 suggests there is relatively less predictive information remaining to be captured by future efforts. If we assume conservative estimates of T1D heritability (e.g. sibling risk ratio of ~6 (14)) are correct then we will have explained the vast majority of T1D heritability with current knowledge and there will be little scope for improvement with future genetic discovery. However, the only way to know this is with future sequencing studies and studies of lower frequency variants that are not well covered in current GWA studies.

As genotyping costs fall and our knowledge of T1D genetic risk increases, additional T1D genetic risk scores will be developed and tested. These might vary in methods of generation, for example where SNPs available for score generation are different, where costs prohibit a full T1D GRS2 to be calculated, or where different populations are used for discovery. It is important to be able to directly compare scores to plan future use and research. A simple but robust approach for this is to compare the variety of published genetic scores for T1D that can be directly compared using score distributions in a reference population. We believe that such direct comparisons will aid the future development and application of T1D genetic risk scores.

Author contributions

RAO, WAH, MNW and SAS designed the study, contributed to analysis and wrote the manuscript. ARW, SEJ, RNB, JHH, DAS, JML and JT contributed to analysis and reviewed the manuscript. SR contributed to study design, provided access to ImmunoChip data from T1DGC, contributed to analysis and reviewed the manuscript. All authors contributed to the discussion and reviewed or edited the manuscript.

Conflicts of interest

RAO holds a UK Medical Research Council institutional Confidence in Concept grant to develop a 10 SNP biochip type 1 diabetes genetic test in collaboration with Randox. The other authors declare no conflict of interest.

Acknowledgements

RAO is supported by a Diabetes UK Harry Keen Fellowship (16/0005529). SS is supported by a Diabetes UK PhD studentship (17/0005757) . WAH is supported by Grant U01DK063829. SR is supported by the National Institute of Health (R01DK096926) and a grant from the University of Virginia Strategic Investment Fund. MNW is supported by the Wellcome Trust Institutional Support Fund (WT097835MF). DS is supported by NIH training grant 2T32DK00724.

This research was performed under the auspices of the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), National Human Genome Research Institute (NHGRI), National Institute of Child Health and Human Development (NICHD), and Juvenile Diabetes Research Foundation International (JDRF). The views expressed are those of the author(s).

Dr. Richard Oram is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT: Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. *Lancet Diabetes Endocrinol* 2018;6:122-129
2. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS: Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA* 2013;309:2473-2479
3. Ziegler AG, Danne T, Dunger DB, Berner R, Puff R, Kiess W, Agiostratidou G, Todd JA, Bonifacio E: Primary prevention of beta-cell autoimmunity and type 1 diabetes - The Global Platform for the Prevention of Autoimmune Diabetes (GPPAD) perspectives. *Mol Metab* 2016;5:255-262
4. Bonifacio E, Beyerlein A, Hippich M, Winkler C, Vehik K, Weedon MN, Laimighofer M, Hattersley AT, Krumsiek J, Frohnert BI, Steck AK, Hagopian WA, Krischer JP, Lernmark Å, Rewers MJ, She JX, Toppari J, Akolkar B, Oram RA, Rich SS, Ziegler AG, Group TS: Genetic scores to stratify risk of developing multiple islet autoantibodies and type 1 diabetes: A prospective study in children. *PLoS Med* 2018;15:e1002548
5. Florez JC: Found in Translation: A Type 1 Diabetes Genetic Risk Score Applied to Clinical Diagnosis. *Diabetes Care* 2016;39:330-332
6. Bingley PJ: Clinical applications of diabetes antibody testing. *J Clin Endocrinol Metab* 2010;95:25-33
7. Landin-Olsson M, Arnqvist HJ, Blohme G, Littorin B, Lithner F, Nystrom L, Schersten B, Sundkvist G, Wibell L, Ostman J, Lernmark A: Appearance of islet cell autoantibodies after clinical diagnosis of diabetes mellitus. *Autoimmunity* 1999;29:57-63
8. de Lusignan S, Sadek N, Mulnier H, Tahir A, Russell-Jones D, Khunti K: Miscoding, misclassification and misdiagnosis of diabetes in primary care. *Diabet Med* 2012;29:181-189
9. Lasserson D, Fox R, Farmer A: Late onset type 1 diabetes. *BMJ* 2012;344:e2827
10. Hope SV, Wienand-Barnett S, Shepherd M, King SM, Fox C, Khunti K, Oram RA, Knight BA, Hattersley AT, Jones AG, Shields BM: Practical Classification

- Guidelines for Diabetes in patients treated with insulin: a cross-sectional study of the accuracy of diabetes diagnosis. *Br J Gen Pract* 2016;66:e315-322
11. Redondo MJ, Jeffrey J, Fain PR, Eisenbarth GS, Orban T: Concordance for islet autoimmunity among monozygotic twins. *N Engl J Med* 2008;359:2849-2850
 12. Pociot F, Lernmark Å: Genetic risk factors for type 1 diabetes. *Lancet* 2016;387:2331-2339
 13. Wray NR, Yang J, Goddard ME, Visscher PM: The genetic interpretation of area under the ROC curve in genomic profiling. *PLoS Genet* 2010;6:e1000864
 14. Clayton DG: Prediction and interaction in complex disease genetics: experience in type 1 diabetes. *PLoS Genet* 2009;5:e1000540
 15. Winkler C, Krumsiek J, Buettner F, Angermüller C, Giannopoulou EZ, Theis FJ, Ziegler AG, Bonifacio E: Feature ranking of type 1 diabetes susceptibility genes improves prediction of type 1 diabetes. *Diabetologia* 2014;57:2521-2529
 16. Oram RA, Patel K, Hill A, Shields B, McDonald TJ, Jones A, Hattersley AT, Weedon MN: A Type 1 Diabetes Genetic Risk Score Can Aid Discrimination Between Type 1 and Type 2 Diabetes in Young Adults. *Diabetes Care* 2016;39:337-344
 17. Perry DJ, Wasserfall CH, Oram RA, Williams MD, Posgai A, Muir AB, Haller MJ, Schatz DA, Wallet MA, Mathews CE, Atkinson MA, Brusko TM: Application of a Genetic Risk Score to Racially Diverse Type 1 Diabetes Populations Demonstrates the Need for Diversity in Risk-Modeling. *Sci Rep* 2018;8:4529
 18. Noble J, Valdes A, Cook M, Klitz W, Thomson G, Erlich H: The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. *Am-J-Hum-Genet* 1996;59:1134-1148
 19. Noble JA: Immunogenetics of type 1 diabetes: A comprehensive review. *J Autoimmun* 2015;64:101-112
 20. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, Mychaleckyj JC, Todd JA, Bonella P, Fear AL, Lavant E, Louey A, Moonsamy P, Type 1 Diabetes Genetics C: HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. *Diabetes* 2008;57:1084-1092
 21. Hu X, Deutsch AJ, Lenz TL, Onengut-Gumuscu S, Han B, Chen WM, Howson JM, Todd JA, de Bakker PI, Rich SS, Raychaudhuri S: Additive and interaction effects at three amino acid positions in HLA-DQ and HLA-DR molecules drive type 1 diabetes risk. *Nat Genet* 2015;47:898-905
 22. Rich SS, Akolkar B, Concannon P, Erlich H, Hilner JE, Julier C, Morahan G, Nerup J, Nierras C, Pociot F, Todd JA: Overview of the Type I Diabetes Genetics Consortium. *Genes Immun* 2009;10 Suppl 1:S1-4
 23. Bycroft C, Freeman C, Petkova D, Band G: Genome-wide genetic data on ~500,000 UK Biobank Participants. *BioRxiv preprint* 2017;
 24. Jia X, Han B, Onengut-Gumuscu S, Chen WM, Concannon PJ, Rich SS, Raychaudhuri S, de Bakker PI: Imputing amino acid polymorphisms in human leukocyte antigens. *PLoS One* 2013;8:e64683
 25. Loh PR, Tucker G, Bulik-Sullivan BK, Vilhjálmsson BJ, Finucane HK, Salem RM, Chasman DI, Ridker PM, Neale BM, Berger B, Patterson N, Price AL: Efficient

- Bayesian mixed-model analysis increases association power in large cohorts. *Nat Genet* 2015;47:284-290
26. Raj P, Rai E, Song R, Khan S, Wakeland BE, Viswanathan K, Arana C, Liang C, Zhang B, Dozmorov I, Carr-Johnson F, Mitrovic M, Wiley GB, Kelly JA, Lauwerys BR, Olsen NJ, Cotsapas C, Garcia CK, Wise CA, Harley JB, Nath SK, James JA, Jacob CO, Tsao BP, Pasare C, Karp DR, Li QZ, Gaffney PM, Wakeland EK: Regulatory polymorphisms modulate the expression of HLA class II molecules and promote autoimmunity. *Elife* 2016;5
 27. Hofmann S, Fischer A, Nothnagel M, Jacobs G, Schmid B, Wittig M, Franke A, Gaede KI, Schürmann M, Petrek M, Mrazek F, Pabst S, Grohé C, Grunewald J, Ronninger M, Eklund A, Rosenstiel P, Höhne K, Zissel G, Müller-Quernheim J, Schreiber S: Genome-wide association analysis reveals 12q13.3-q14.1 as new risk locus for sarcoidosis. *Eur Respir J* 2013;41:888-900
 28. Onengut-Gumuscu S, Chen WM, Burren O, Cooper NJ, Quinlan AR, Mychaleckyj JC, Farber E, Bonnie JK, Szpak M, Schofield E, Achuthan P, Guo H, Fortune MD, Stevens H, Walker NM, Ward LD, Kundaje A, Kellis M, Daly MJ, Barrett JC, Cooper JD, Deloukas P, Type 1 Diabetes Genetics C, Todd JA, Wallace C, Concannon P, Rich SS: Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. *Nat Genet* 2015;47:381-386
 29. Howson JM, Rosinger S, Smyth DJ, Boehm BO, Group A-ES, Todd JA: Genetic analysis of adult-onset autoimmune diabetes. *Diabetes* 2011;60:2645-2653
 30. Redondo MJ, Geyer S, Steck AK, Sharp S, Wentworth JM, Weedon MN, Antinozzi P, Sosenko J, Atkinson M, Pugliese A, Oram RA, Group TDTs: A Type 1 Diabetes Genetic Risk Score Predicts Progression of Islet Autoimmunity and Development of Type 1 Diabetes in Individuals at Risk. *Diabetes Care* 2018;
 31. Usher-Smith JA, Thompson MJ, Sharp SJ, Walter FM: Factors associated with the presence of diabetic ketoacidosis at diagnosis of diabetes in children and young adults: a systematic review. *BMJ* 2011;343:d4092
 32. Noble JA, Erlich HA: Genetics of type 1 diabetes. *Cold Spring Harb Perspect Med* 2012;2:a007732
 33. Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, Natarajan P, Lander ES, Lubitz SA, Ellinor PT, Kathiresan S: Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet* 2018;50:1219-1224
 34. Redondo MJ, Oram RA, Steck AK: Genetic Risk Scores for Type 1 Diabetes Prediction and Diagnosis. *Curr Diab Rep* 2017;17:129
 35. Brody JA, Morrison AC, Bis JC, O'Connell JR, Brown MR, Huffman JE, Ames DC, Carroll A, Conomos MP, Gabriel S, Gibbs RA, Gogarten SM, Gupta N, Jaquish CE, Johnson AD, Lewis JP, Liu X, Manning AK, Papanicolaou GJ, Pitsillides AN, Rice KM, Salerno W, Sitlani CM, Smith NL, Heckbert SR, Laurie CC, Mitchell BD, Vasan RS, Rich SS, Rotter JI, Wilson JG, Boerwinkle E, Psaty BM, Cupples LA, Consortium NT-OfPMT, Consortium CfHaARiGEC, Group THaHW, Group CAaBW: Analysis commons, a team approach to discovery in a big-data environment for genetic epidemiology. *Nat Genet* 2017;49:1560-1563

Figures and Table Legends

Figure 1- Manhattan plots of GWAS before and after adjustment for GRS: Top row, genome-wide GWAS: Panel A, unadjusted; Panel B, after adjusting for GRS1; Panel C after adjustment for GRS2. Bottom row, high resolution plot of association scores for the full HLA region on Chr 6q: Panel D, unadjusted; Panel E, after adjustment for GRS1; Panel F, after adjustment for GRS2.

Figure 2- ROC curves comparing the ability of GRS1 and GRS2, and their components, to discriminate T1D. For each pair of curves in a panel, the figures show ROC AUCs and the p-value for their comparison by the DeLong algorithm.

A HLA DR-DQ haplotypes and interaction terms, for GRS1 vs GRS2 in the T1DGC.

B Both non-DR-DQ HLA and non-HLA loci, for GRS1 vs GRS2 in the T1DGC.

C Full GRS1 versus full GRS2 in the T1DGC discovery dataset.

D HLA DR-DQ haplotypes and interaction terms, for GRS1 vs GRS2 in the UK Biobank.

E Both non-DR-DQ HLA and non-HLA loci, for GRS1 vs GRS2 in UK Biobank.

F Full GRS1 versus full GRS2 in UK Biobank.

Figure 3- Comparison of the ability of GRS1 (left) and GRS2 (right) to discriminate type 1 diabetes (red) from controls (blue) in 3A and 3B respectively. Comparison of the ability of GRS1 (left) and GRS2 (right) to discriminate type 1 diabetes (red) from

type 2 diabetes (yellow) in 3C and 3D respectively. Note the similar distribution of T1D GRS2 scores in the background population and in T2D cases.

Table 1- Simulated population-based prediction of T1D using HLA screening, the original T1D GRS, and the T1D GRS2. Risk of T1D is calculated assuming a 0.3% population prevalence of T1D.

T1D Centile*	Population Centile**	GRS2	Specificity	Sensitivity	1-Spec	Youden's Index (j)	T1D risk***
5	70.2	11.68	69.5%	94.8%	30.5%	0.643	0.9%
10	79.4	12.36	78.9%	89.4%	21.1%	0.683	1.3%
25	90.6	13.45	90.4%	77.5%	9.6%	0.679	2.4%
50	96.8	14.60	96.7%	53.7%	3.3%	0.505	4.7%
75	99.1	15.65	99.1%	30.2%	0.9%	0.293	9.1%
90	99.8	16.54	99.8%	13.2%	0.2%	0.130	15.7%
95	99.9	17.06	99.9%	7.2%	0.1%	0.072	22.8%

T1D Centile*	Population Centile**	GRS1	Specificity	Sensitivity	1-Spec	Youden's Index (j)	T1D risk***
5	53.3	13.48	51.9%	95.9%	48.1%	0.478	0.6%
10	60.7	14.06	63.9%	92.5%	36.1%	0.564	0.8%
25	73.8	15.07	81.7%	82.9%	18.3%	0.646	1.3%
50	86.1	16.16	94.0%	56.3%	6.0%	0.503	2.7%
75	93.3	17.17	98.3%	32.6%	1.7%	0.308	5.4%
90	96.5	17.83	99.5%	18.3%	0.5%	0.178	9.9%
95	97.8	18.19	99.8%	9.0%	0.2%	0.088	11.8%

T1D Centile*	Risk Category	HLA Type	Specificity	Sensitivity	1-Spec	Youden's Index (j)	T1D risk***
-	Background	Other	0.0%	100.0%	0.0%	0.000	0.3%
57.0	Moderate	DR3/3, DR4/X	79.1%	77.0%	23.0%	0.561	0.6%
81.1	High	DR4/4	96.3%	41.3%	58.7%	0.376	2.5%
84.5	Very High	DR3/4	97.2%	37.0%	63.1%	0.342	3.8%

* T1D Cases in T1DGC

** Centile in UK Biobank European population